

Docket No. UCT-0039

Claims

1. (Original) A purified or isolated Herpes simplex virus recombinase comprising an alkaline nuclease and a single stranded DNA binding polypeptide, wherein the recombinase has polynucleotide strand exchange activity.
2. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 1, comprising a Herpes simplex virus-1 recombinase.
3. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 2, wherein the alkaline nuclease is Herpes simplex virus-1 UL12 and the single stranded DNA binding polypeptide is Herpes simplex virus-1 ICP8.
4. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 2, wherein the ratio of the alkaline nuclease to the single stranded DNA binding polypeptide is 1:500 to 1:1.
5. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 3, wherein the alkaline nuclease, the single stranded DNA binding polypeptides, or both are isolated polypeptides.
6. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 3, wherein the alkaline nuclease, the single stranded DNA binding protein, or both are expressed in a host cell.
7. (Original) The purified or isolated Herpes simplex virus recombinase of Claim 6, wherein the host cell is an insect cell or a VERO cell.
8. (Original) A host cell comprising a Herpes simplex virus recombinase, wherein the Herpes simplex virus recombinase is expressed from a first polynucleotide comprising a Herpes simplex virus-1 UL12 polynucleotide operatively linked to expression control sequences, and a second polynucleotide comprising a Herpes simplex virus-1 ICP8 polynucleotide operatively linked to expression control sequences.

Docket No. UC1-0039

9. (Original) The host cell of Claim 8, wherein the first polynucleotide and the second polynucleotide are present on a single expression vector.

10. (Original) The host cell of Claim 8, wherein the host cell is an insect cell or a VERO cell.

11. (Original) A method of promoting homologous recombination, comprising contacting:

a purified or isolated Herpes simplex virus recombinase, wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide, and wherein the recombinase has polynucleotide strand exchange activity;

a donor polynucleotide comprising a first donor homology region at a first end, a second donor homology region at a second end, and an exogenous sequence therebetween; and

a target polynucleotide comprising a first donor homology region at a first end, a second donor homology region at a second end, and an endogenous sequence therebetween;

wherein contacting is performed under conditions sufficient to promote homologous recombination.

12. (Original) The method of Claim 11, wherein the first donor homology region and the first target homology region are substantially homologous; and wherein the second donor homology region and the second target homology region are substantially homologous.

13. (Original) The method of Claim 11, wherein contacting is *in vitro*.

14. (Original) The method of Claim 13, wherein the alkaline nuclease comprises purified Herpes simplex virus-1 UL12 and the single stranded DNA binding polypeptide comprises purified herpes simplex virus-1 ICP8.

15. (Original) The method of Claim 11, wherein contacting is in a host cell.

16. (Original) The method of Claim 15, wherein the host cell is a mammalian cell.

Docket No. UCT-0039

17. (Original) The method of Claim 15, wherein the host cell comprises a first polynucleotide comprising a Herpes simplex virus-1 UL12 polynucleotide operatively linked to expression control sequences, and a second polynucleotide comprising a Herpes simplex virus-1 ICP8 polynucleotide operatively linked to expression control sequences.

18. (Original) A cloning kit, comprising:

a Herpes simplex virus recombinase, wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide, and wherein the recombinase has polynucleotide strand exchange activity; and

a target polynucleotide comprising a first homology region at a first end, a second homology region at a second end, and an endogenous sequence therebetween.

19. (Original) The cloning kit of Claim 18, wherein the Herpes simplex virus recombinase comprises a Herpes simplex virus-1 recombinase.

20. (Original) The cloning kit of Claim 19, wherein the alkaline nuclease is Herpes simplex virus-1 UL12 and the single stranded DNA binding polypeptide is Herpes simplex virus-1 ICP8.

21. (Original) The cloning kit of Claim 18, further comprising a host cell.

22. (Original) The cloning kit of Claim 21, wherein the host cell comprises a first polynucleotide comprising a Herpes simplex virus-1 UL12 polynucleotide operatively linked to expression control sequences, and a second polynucleotide comprising a Herpes simplex virus-1 ICP8 polynucleotide operatively linked to expression control sequences.

23. (Original) The cloning kit of Claim 18, wherein the endogenous sequence comprises a polylinker.

24. (Original) The cloning kit of Claim 18 wherein the endogenous sequence comprises at least one regulatory sequence for protein expression.

Docket No. UCT-0039

25. (Original) A method of treating a eukaryotic host cell, comprising delivering to the eukaryotic host cell:

a Herpes simplex virus recombinase, wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide, and wherein the recombinase has polynucleotide strand exchange activity; and

a donor polynucleotide comprising a first donor homology region at a first end, a second donor homology region at a second end, and an exogenous sequence therebetween.

26. (Original) The method of Claim 25, wherein the Herpes simplex virus recombinase comprises a Herpes simplex virus-1 recombinase.

27. (Original) The method of Claim 26, wherein the alkaline nuclease is Herpes simplex virus-1 UL12 and the single stranded DNA binding polypeptide is Herpes simplex virus-1 ICP8.

28. (Original) A method of obtaining a transgenic non-human animal, comprising: delivering to an embryonic stem cell or zygote a Herpes simplex virus recombinase, wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide; and a donor polynucleotide comprising a first homology region at a first end, a second homology region at a second end, and an exogenous sequence therebetween; wherein the exogenous sequence integrates into a genome of the embryonic stem cell or the zygote; and

producing from the embryonic stem cell or the zygote a transgenic non-human animal.

29. (Original) The method of Claim 28, wherein the Herpes simplex virus recombinase comprises a Herpes simplex virus-1 recombinase.

30. (Original) The method of Claim 29, wherein the alkaline nuclease is Herpes simplex virus-1 UL12 and the single stranded DNA binding polypeptide is Herpes simplex virus-1 ICP8.

Docket No. UCT-0039

31. (Original) The method of Claim 28, wherein the transgenic animal comprises a gene knock-out.

32. (Original) A method of treating an organism comprising:
delivering to the organism a composition comprising a Herpes simplex virus recombinase; and a donor polynucleotide comprising a first homology region at a first end, a second homology region at a second end, and an exogenous sequence therebetween; wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide, and wherein the recombinase has polynucleotide strand exchange activity.

33. (Original) The gene therapy method of Claim 32, wherein the Herpes simplex virus recombinase comprises a Herpes simplex virus-1 recombinase.

34. (Original) The method of Claim 32, wherein the Herpes simplex virus recombinase is expressed in an infectious vector.

35. (Original) A method of making a modified host cell comprising:
delivering to the host cell a composition comprising a Herpes simplex virus recombinase; and a donor polynucleotide comprising a first homology region at a first end, a second homology region at a second end, and an exogenous sequence therebetween; wherein the Herpes simplex virus recombinase comprises an alkaline nuclease and a single stranded DNA binding polypeptide, and wherein the recombinase has polynucleotide strand exchange activity.

36. (Original) The method of Claim 35, wherein the Herpes simplex virus recombinase comprises a Herpes simplex virus-1 recombinase.